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Transplacental Immunity of Varicella Zoster Virus and Factors Affecting it Versus Immunity in Preschool and School Children

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This study aimed to evaluate prevalence of varicella zoster virus immunoglobulin-G (VZV-IgG) in neonatal and maternal serum, comparing it with seroprevalence in preschool and school children and to assess influence of gestational age, birth weight and parity on it, besides the predictive value of history of previous varicella infection. We selected 88 subjects classified into 4 groups. Groups I and II consisted of 20 mother-newborn pairs. Group III consisted of 13 children (1-4 years) and Group IV consisted of 35 children (5-16 years). Determining VZV-IgG using ELISA technique was done. We found 60.2% of overall subjects seropositive for VZV-IgG. Prevalence in different groups was 50%, 45%, 23.1% and 88.6%, respectively (p=0.0001). There was no sex related difference (p>0.05).

Multipara mothers had higher VZV-IgG seroprevalence than primipara (p=0.019). Full term neonates had significantly higher VZV-IgG seroprevalence than prematures (p=0.04). Children of group III with history of varicella infection were all VZV-IgG seropositive and those with no history were seronegative (p=0.0001).

In group IV, children with history of varicella infection were all VZV-IgG seropositive, while in children with no history of varicella infection four were VZV-IgG seronegative and three were seropositive (p=0.0001). Positive predictive value of history of varicella infection of groups III and IV was 100% and negative predictive value was 82.35% with 100% specificity and 91.18% sensitivity. We concluded that, significant proportions of neonates, preschool children and mothers were susceptible to varicella. Prematurity might influence maternally acquired VZV-IgG. Positive history of varicella infection could be a reliable marker of VZV-IgG seroconversion.

Key words: Varicella zoster, neonates, premature, low birth weight, prevalence, vaccination

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INTRODUCTION

Varicella (chicken pox) is a mild, very contagious and vaccine preventable disease, occurring mostly during childhood, caused by Varicella Zoster Virus (VZV), a ubiquitous human α-herpesvirus (Myers et al., 2004).

Although it usually presents a benign course and is usually self-limiting disease in otherwise healthy children, it may cause serious complications and even mortality in adolescents, adults, pregnant women and especially in high risk individuals including immuno-compromised persons, premature (PT) and critically ill neonates (Friedman et al., 1994; Centers for Disease Control, 1996; American Academy of pediatrics, 2003).

During pregnancy, protective maternal antibodies are transferred to the fetus, mainly in the third trimester and therefore, PT neonates may not have acquired enough maternal antibodies to be protected (Prevention of varicella, 1996). Anti-VZV titers decrease to undetectable levels in PT infants by 6 months of age or earlier; thus these infants appear to be susceptible to chickenpox (Linder et al., 2000; Leineweber et al., 2004).

In tropical countries, a greater proportion of adolescents and adults are susceptible to varicella and with higher risk of complications, compared with those of temperate countries (American academy of Pediatrics, 2003).

The epidemiology of severe varicella infections in pediatric patients aged 0-16 years and its complications and mortality especially of infants describes a sizeable hospitalization and complication rate in many countries and provides a solid basis for future immunization recommendations. It has been suggested that the pattern of primary infection with VZV has changed in recent years (Meyer et al., 2000; Kudesia et al., 2002; Bonhoeffer et al., 2005; Nguyen, 2005).

Varicella is uncommon in women during pregnancy. If, however, maternal varicella infects the fetus, intrauterine death or severe diseases (congenital or fetal varicella syndrome) may ensue depending on the time of maternal infection. The risk of infection may be greater for susceptible parous women. Approximately 6% of women of childbearing age are probably not immune to VZV (Coyle et al., 1997; Sauerbrei, 1998; Kudesia et al., 2002).

At present, 50% of all VZV-immunoglobulin (IgG) issued for passive prophylaxis is for pregnant contacts and neonates and other high risk subjects with significant exposure to varicella zoster (Miller et al., 1996; Litt and Burgess, 2003).

The documented decline in varicella mortality for children, adults and all racial and ethnic groups support the success of the current vaccination program applied in many developed countries and should be useful for countries considering a universal varicella vaccination program. However, infants are not eligible for vaccination (Nguyen, 2005).

The prevalence of varicella infection in Egypt is not clear because it is not an obligatory reported disease and there are not enough epidemiologic studies of VZV-IgG seroprevalence. Routine vaccination against varicella is not currently practiced in Egypt.

The aim of this study was to evaluate the prevalence of varicella zoster virus immunoglobulin-G (VZV-IgG) in neonatal and maternal serum, then comparing it with seroprevalence in preschool and school children and to assess the effect of gestational age, birth weight and parity on it, in addition to the predictive value of history of previous varicella infection.

MATERIALS AND METHODS

Eighty eight healthy subjects were enrolled in this study in the form of 4 groups. Groups I and II consisted of 20 mother-newborn pairs who were selected from the labor division of Ahmed Maher Teaching Hospital.

Mothers' ages ranged from 19-39 years old. Women who had received a blood transfusion during pregnancy were excluded. Basic demographic data and antenatal care were documented. Clinical and obstetric history, last menstrual period, parity, medication used during pregnancy and immunization history were obtained from the women's antenatal health card, if available; otherwise by direct questions, after obtaining maternal consent. None of them had varicella infection during pregnancy. Complete physical examination was done including weight and height. The mode of delivery was recorded.

Neonates were 10 males and 10 females with a mean gestational age of 37.7±2.13 weeks. On delivery, each baby was resuscitated and was assessed by using the Apgar scoring system and a clinical examination was done. Each baby was weighed and crown-heel length was measured. Gestational ages were determined from the last menstrual period of the mother and confirmed by using Dubowitz score (Dubowitz et al., 1970).

Groups III and IV were selected from the Pediatric Clinic of the National Research Center. Group III consisted of 13 children with age range 1-4 years, while group IV consisted of 35 children with age range 5-16 years. Infants aged less than 1 year were excluded because it would not be possible to distinguish passively acquired maternal antibody from that resulting from infection. Complete physical examination was done including weight and height. After informed consent had been obtained, parents were asked whether their child had a history of varicella infection or not.
None of the subjects included in the study was vaccinated for varicella zoster.

**Laboratory investigations**

- Complete blood count (CBC).
- Serum level of Anti-VZV-IgG antibodies by enzyme-linked immunosorbsent assay (ELISA).

Maternal blood (3 mL) was obtained from a peripheral vein just after delivery. Cord blood (3 mL) was collected from large veins on the fetal side of the placenta immediately after delivery. Venous blood samples (3 mL) were withdrawn from children of group III and IV on plane tubes. Serum samples separated after centrifugation were stored at -20°C until analyzed.

**Assessment of IgG class antibodies to varicella-zoster virus (VZV):** Qualitative immunoenzymatic determination of IgG-class antibodies against Varicella-Zoster Virus (VZV) was based on the ELISA (Enzyme-linked Immunosorbent Assay) technique, by using NovaTec Immunodiagnostics GmbH Technologie and Waldpark, Waldstr. 23 A6, D-63128 Dietzenbach, Germany, Prod. No. VZVG 0490, VZV-IgG ELISA (96 Determinations).

Microtiter strip wells are precoated with VZV antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseadish peroxidase labelled antihuman IgG conjugate is added. This conjugate binds to the captured VZV-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of VZV-specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450 nm is read using an ELISA microwell plate reader. Values above the cut-off point were considered to be positive, indicating previous exposure to VZV infection (Groen and Straus, 1991).

**Statistical methods:** Statistical Package for Social Sciences (SPSS) program version 11 was used for analysis of data. Data were described in terms of mean±SD and percentage. Comparison between two groups was done by using Student’s t-test for quantitative independent variables and Chi square test for qualitative variables. Mann-Whitney test was used when Student’s t-test was not applicable. ANOVA test was used for analysis of more than two groups followed by post Hoc test if significant. P-value is considered significant if < 0.05.

**RESULTS**

This study included 88 subjects who were classified into 4 groups. Group I consisted of 20 mothers with a mean age of 24.65±5.53 years and age range 19-39 years. Seven mothers (35%) were primipara and 13 (65%) were multipara. Eleven (55%) mothers gave birth through normal vaginal delivery (NVD), while caesarean section (CS) was done for 9 (45%) mothers. Hemoglobin (Hb) level was lower in mothers with CS compared to those with NVD (9.97±1.86 and 11.65±1.72, respectively) (p = 0.048).

Group II consisted of 20 neonates of the preceding mothers (10 males and 10 females) with a mean gestational age of 37.7±2.13 weeks. Neonates were classified into several subgroups according to gestational age, birth weight and mode of delivery as follow: Full term neonates (FT) (>37 weeks gestational age), premature neonates (PT) (<37 weeks gestational age), adequate birth weight neonates (ABW) (>2500 g), low birth weight neonates (LBW) (<2500 g), neonates born by NVD and those born by CS. Hb level was statistically significantly lower in PT, LBW and neonates born by CS compared to FT, ABW and neonates born vaginally (p = 0.01, p = 0.01 and p = 0.001, respectively).

Group III consisted of 13 children (6 males and 7 females) with a mean age of 3.41±0.62 years and age range 1-4 years.

Group IV consisted of 35 children (20 males and 15 females) with a mean age of 10.32±2.22 years and age range 5-16 years.

All mothers of group I were not sure whether they had a history of varicella infection or not in childhood and none of them had varicella infection during pregnancy. Three children of group III (23.1%) had a history of varicella infection, while 28 (80%) children of group IV had a history of previous varicella infection.

Table 1 demonstrates clinical and laboratory data of the 4 groups included in the study.

Out of overall subjects included in our study 60.2% of them were seropositive for VZV-IgG. Seropositivity in the different groups were 50% in group I, 45% in group II, 23.1%, in group III and 88.6% in group IV respectively. A statistically significant difference was found among the 4 groups, where VZV-IgG seroprevalence was significantly higher in group IV than the other three groups (p = 0.0001) (Fig. 1). There was no significant sex related difference concerning VZV-IgG seropositivity (p>0.05).

There was a statistically significant difference of VZV-IgG seropositivity between primipara and multipara mothers [1/7 (14.3%) and 9/13 (69.2%) respectively] (p = 0.019).
Table 1: Clinical and laboratory data of different groups included in the study

<table>
<thead>
<tr>
<th>Items</th>
<th>Group I (N = 20)</th>
<th>Group II (N = 20)</th>
<th>Group III (N = 13)</th>
<th>Group IV (N = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female (N. and %)</td>
<td>0/20</td>
<td>10/10</td>
<td>6/7</td>
<td>20/15</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>24.6±5.53</td>
<td>3.7±0.62</td>
<td>10.3±2.22</td>
<td>18.4±3.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.5±12.96</td>
<td>3.12±0.76</td>
<td>17.23±3.85</td>
<td>29.73±10.57</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.6±5.77</td>
<td>40.75±3.69</td>
<td>103.69±9.40</td>
<td>130.03±15.71</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.89±1.94</td>
<td>14.54±1.85</td>
<td>12.47±0.92</td>
<td>11.62±1.40</td>
</tr>
</tbody>
</table>

Total leukocyte count (10³/mm³)

<table>
<thead>
<tr>
<th>Items</th>
<th>Group III (N = 13)</th>
<th>Group IV (N = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of chickenpox</td>
<td>Yes 0 vs No 28</td>
<td>Yes 0 vs No 3</td>
</tr>
<tr>
<td>History of chickenpox</td>
<td>Yes 10 vs No 4</td>
<td>Yes 0 vs No 4</td>
</tr>
</tbody>
</table>

*p-value is significant if <0.05

Table 2: Comparison of history of varicella infection and varicella zoster virus serology in groups III and IV

Data were expressed as Mean±SD, except for numbers between parentheses

Figure 2 shows the percentages of VZV-IgG seropositivity among the different neonatal subgroups. As we see, although the percentages of seropositivity were higher in FT than PT subgroup, in ABW than LBW subgroup and in neonates born vaginally versus those born by CS, yet, a statistically significant difference was only found between FT and PT neonates (p<0.05).

All children of group III who had a history of varicella infection (3/13) were seropositive for VZV-IgG and those who had no history (10/13) were all seronegative (p=0.0001). On the other hand, out of the 35 children of group IV, 28 who had a history of varicella infection were found to be seropositive for VZV-IgG, while out of the seven children who had no history of varicella infection four were seronegative for VZV-IgG and three were seropositive (p=0.0001) (Table 2).

Positive predictive value of history of varicella infection of groups III and IV was 100% and negative predictive value was 82.35% with 100% specificity and 91.18% sensitivity (Table 3).

**DISCUSSION**

Since the implementation of the varicella vaccination program in the United States, varicella-related deaths have declined dramatically to the lowest level among children and adults and are considerably lower than the reported rates in countries that do not have a universal vaccination program. Varicella vaccine coverage among children 19 to 35 months of age has markedly increased in the past years (Fairley and Miller, 1996; Chant et al., 1998; Bramley and Jones, 2000; Rawson et al., 2001; NSUAVC, 2002; Nguyen, 2005).

In our study there was no significant difference between mothers and neonates regarding the percentage of VZV-IgG seropositivity which was in agreement with van Der Zwen et al. (2002) who mentioned that, the neonatal VZV-IgG titer is predominantly predicted by the maternal VZV-IgG titer.

Varicella IgG seropositivity was statistically significantly higher in multipara mothers than primipara which might be due to an increased risk of exposure to infected children in the home, which coincides with Coyle et al. (1997) who mentioned that, the risk of infection may be greater for susceptible parous women.
The present study revealed that prevalence of VZV-IgG seropositivity was statistically significantly higher in FT than PT neonates and a significant positive association between it and gestational age was found which was in agreement with what was reported by Wesumperuma et al. (1999), who added that, placental antibody transfer to VZV antibodies was significantly lower in PT than PT neonates. This was explained later by the fact that, the fetus receives protective maternal VZV-IgG mainly in the third trimester of pregnancy. Therefore, PT neonates are considered at risk for VZV infection (Van Der Zwet et al., 2002).

So, to prevent deaths from varicella among high-risk conditions, immunity insurance through the vaccination of close contacts and administering VZV-IgG to exposed PT neonates within 96 h of exposure is recommended to prevent severe disease and subsequent complications, as infants are not eligible for vaccination (Prevention of varicella, 1996, 1999; van Der Zwet et al., 2002; Nguyen, 2005).

Our study also showed that, although seroprevalence of VZV-IgG was higher in ABW neonates compared with values of LBW neonates, still, the difference was not statistically significant. This coincides with van Der Zwet et al. (2002) who reported that, the neonatal VZV-IgG titer is predominantly predicted by the maternal VZV-IgG titer, whereas birth weight is much less predictive than previously reported. Other investigators beforehand found that LBW was independently associated with significantly lower levels of antibody transfer for VZV (Wesumperuma et al., 1999; Okoko et al., 2001).

Additional analysis of group II illustrated that the mode of delivery had an influence on VZV-IgG seroprevalence as it was higher in neonates born by NVD than those born via CS and the difference almost approached significance. This might be explained by the fact that 77.8% of the neonates born by CS in our study were premature. Moreover, all LBW neonates included in our study were delivered by CS.

We found that Hb level was significantly lower in PT, LBW and also in neonates born via CS. The cause of this might be attributed to the fact that, mothers incorporated in our study who gave birth through CS were anemic compared to mothers who gave birth vaginally with subsequent reflection on Hb level of their offspring where the majority of the neonates who were born by CS were PT and of LBW. Previous investigators reported that, maternal anemia influences birth weight and preterm delivery and was found to be significantly associated with them. In addition, higher rates of CS were found among anemic women (El Guindi et al., 2004; Levy et al., 2005).

The study demonstrated a statistically significant difference among the four groups included in our study as regards VZV-IgG seroprevalence, whereas it was significantly higher in group IV (5-16 years) than the other three groups.

Seroprevalence of VZV-IgG was lower in mothers (group I) than group IV which might be due to less exposure to previous chickenpox infection in childhood. Kudesa et al. (2002) reported that prevalence of serum VZV-IgG declined marginally in age group 20-29 years with no significant change in other age groups of their study.

Maternal serum level of VZV-IgG consequently was reflected on their neonates (group II) through transplacental passive transfer of VZV-IgG during the last trimester of pregnancy as the newborn’s capacity to produce antibodies (IgG) is relatively low during first months of life (De Moraes-Pinto and Hart, 1997), with subsequent lower VZV-IgG seroprevalence as well in group II when compared to group IV.

Moreover, the higher VZV-IgG seroprevalence in group IV as compared to group III (1-4 years) might be attributed to fading of the maternally acquired VZV-IgG in group III which is supported by what was reported in the study of Vazquez et al. (2001) that the time of the disappearance of maternal antibodies is between 12 and 18 months and he recommended vaccination after it. Besides, only 23.1% of children of this group had a history of chickenpox infection, which might be due to less exposure to clinical chickenpox infection since those preschool children were not attending creches, nurseries or day care centers. On the other hand, 80% of children of group IV had a history of previous chickenpox infection, as a result of more exposure at schools, nurseries and day care centers. It has been reported that 14 and 16% of children attending day care centers in the USA and France develop chickenpox, respectively (Saddier et al., 1998; Vazquez et al., 2001).

Several studies reported an increase in the incidence of clinical chickenpox in the 1-4 year age group although before 1982 the highest incidence of clinical chickenpox was in 5-14 years old, which means a shift in clinical incidence, probably because of early exposure of preschool children in developed countries in creches and nurseries (Joseph and Noah, 1988; Yawn, 1997; Saddier et al., 1998; Vazquez et al., 2001; Kudesa et al., 2002).

Vyse et al. (2004) mentioned that prevalence of VZV rise rapidly with age, with 53% of children showing evidence of prior infection by the age of 5 years and most young adults having experienced infection.
Previous studies have suggested that the pattern of primary infections with VZV has changed in recent years, with an upward shift in age distribution. Monitoring of cases has shown a significant increase in both the absolute number and the proportion of cases in those over 14 years in the past 20 years (Sloan and Burlinson, 1992; Miller et al., 1993; Kudesia et al., 2002).

Our study revealed that there was no significant sex related difference concerning VZV-IgG seroprevalence which was in agreement with Savas et al. (2004) and Ozkan et al. (2005).

We found that, 60.2% of subjects included in the current study were seropositive for VZV-IgG antibodies. This was consistent with Heininger et al. (2005) who found that overall, 61% of cases of their study were positive for VZV antibodies. Furthermore, seroprevalence in their different age groups was almost comparable to our results. Also, our results were found to be more or less similar to what was reported by Ozkan et al. (2005).

Our results of maternal varicella IgG seroprevalence showed that 50% of them were susceptible to varicella infection. Their seroprevalence is lower than that reported by other investigators. Alonen et al. (2005) stated that seroprevalence of varicella IgG were 96.2% in maternal serum. Also, Knowles et al. (2004), stated that across different worldwide regions, 6.9% of Irish and other Western European women were susceptible to VZV. They added that, there are important differences in immunity to these infections and so of potential risk of an adverse outcome.

So, if mothers got chickenpox infection which is uncommon during pregnancy, the highest risk being when maternal infection occurs between 13-20 weeks gestation, where congenital anomalies may occurred (Litt and Burgess, 2003).

Goldberg et al. (2002) stated that obstetric populations born in tropical or subtropical regions are likely to be seronegative for varicella virus and those regions will benefit greatly from being vaccinated for varicella immunity. Seidman et al. (1996) reported that targeting women of reproductive age group, especially in a postpartum program, may be an effective vaccination strategy. Some investigators suggest that, postpartum vaccination of varicella-susceptible women need not be delayed because of breast-feeding as they found no evidence of varicella vaccine virus excretion in breast milk (Bohike et al., 2003).

Positive history of varicella infection was an excellent predictor of VZV-IgG seropositivity in groups III and IV with positive predictive value 100% and negative predictive value 82.35%, with 100% specificity and 91.18% sensitivity. This coincided with many investigators who found that patients' history of varicella had a high positive predictive value and a low negative predictive value especially in children and adolescents. They suggested that a positive history of varicella is a reliable marker of disease while a negative history does not predict lack of immunity (Heininger et al., 2005; Holmes, 2005; Lafer et al., 2005).

So, serologic test is advised, rather than presumptive vaccination, for persons with a negative or doubtful history of varicella. For those with a positive history of varicella at higher risk of varicella infection (e.g., pregnant women), serum testing is recommended. For low risk people, a positive history of varicella could be accepted as a reliable indicator of immunity.

WHO (1998) did not recommend the inclusion of varicella vaccination into the routine immunization programs of developing countries. This was attributed to the fact that, in most developing countries, other new vaccines, including hepatitis B vaccine, have a greater public health impact and should therefore be given priority over varicella vaccine.

Varicella vaccine may be used either at an individual level to protect susceptible adolescents and adults, or at a population level, to cover all children as part of a national immunization program. Vaccination of adolescents and adults will protect at-risk individuals, but will not have a significant impact on the epidemiology of the disease. On the other hand, extensive use as a routine vaccine in children will have a significant impact on the epidemiology of the disease. Moreover, it may affect the frequency and the severity of herpes zoster. Varicella vaccine may be used for postexposure prophylaxis and is most effective if given within three days after exposure, but can be used up to five days from exposure. However, infants and the majority of immunocompromised persons are not eligible for vaccination (Prevention of varicella, 1996, 1999; WHO, 1998; Frederiksen et al., 2003; Litt and Burgess, 2003 and Nguyen, 2005).

CONCLUSIONS AND RECOMMENDATIONS

The present study showed that a significant proportion of neonates, preschool children and adult mothers are susceptible to varicella.

Prematurity may influence the level of maternally acquired immunity to VZV-IgG and may further predispose these already vulnerable neonates to viral infections. So, alternative preventive strategies are needed to provide better protection to those susceptible infants. Causes of preterm labor should be avoided including maternal anemia which should be treated early in pregnancy.
The positive predictive value of history of varicella infection is high in preschool and school children and so, could be a reliable marker of seroprevalence of varicella IgG and except for subjects who are at increased risk of varicella infection or its complications; sero testing may not be required but is advised for those with negative history before selective immunization in countries where universal immunization against varicella is not applied as

If varicella vaccine was to be introduced as a universal childhood vaccine in Egypt, then, for the vaccination program to be effective, vaccine should be targeted at an age group between 12 and 18 months; that is soon after the disappearance of maternally acquired immunity, in addition to susceptible women in the reproductive period after sero testing.

Finally, a nationwide seroepidemiological and disease surveillance study is recommended and should be designed on a wide range, in addition to evaluating the clinical impact of varicella and the cost-benefits before the introduction of varicella vaccination into the routine childhood immunization schedule in Egypt in the future.

REFERENCES


